MANURE MANAGEMENT

Differential Nitrogen-15 Labeling of Dairy Manure Components for Nitrogen Cycling Studies

J. Mark Powell,* Zhiguo Wu, Keith Kelling, Paul Cusick, and Gabriela Muñoz

ABSTRACT

Current estimates of dairy manure nitrogen availability to crops are based on indirect measures and vary greatly. The objective of this study was to differentially label dairy manure N components with the stable isotope ^{15}N for direct measurement of manure N cycling in soils and availability to crops. Dairy urine and fecal N components (microbial and undigested feed N) were differentially labeled by feeding either ^{15}N-enriched forage or urea to mature dry dairy cows (*Bos taurus*). Nitrogen-15–enriched ammonium sulfate was used to label alfalfa (*Medicago sativa* L.) hay and corn (*Zea mays* L.) silage. These ^{15}N-enriched forages or either single or multiple doses of ^{15}N-enriched urea were fed for 2 to 3 d, and feces and urine were collected separately for 8 d after the initiation of ^{15}N feeding. For both labeling techniques, ^{15}N appeared first in urine followed by fecal microbial and undigested feed N. For the forage method, the proportionate combination of feces excreted before and after peak ^{15}N excretion levels would achieve uniform labeling of fecal N components. For the urea method, no undigested feed N in feces was labeled since ^{15}N-enriched forage N was not fed. The choice of which labeling method to use depends on the intended use of labeled manure. Manure enriched using the forage method and high levels of manure ^{15}N enrichments should be used for long-term manure N cycling studies. Manure enriched using the urea method and lower ^{15}N enrichments could be used in shorter-term studies.

Over the past 25 yr, the fertilizer value of dairy manure has become less appreciated as the use of inexpensive, high-analysis and custom-blend fertilizers have become widespread. On many dairy farms, manure has become an undesirable by-product of milk production, and most connotations of its intrinsic fertilizer value have been replaced with an animal waste mentality. For example, when calculating fertilizer application rates for field crops, many dairy farmers do not credit the nutrients they applied in the form of manure (Nowak et al., 1997). This may be due to many factors, including a perception that manure is an undependable nutrient source.

Manure nutrient availability to crops is estimated by indirect methods and varies widely. For example, using the *difference method* and the *fertilizer equivalent approach*, 12 to 63% of dairy manure N may be taken up by corn during the first growing season after application (Motavalli et al., 1989; Klausner et al., 1994). Nutrient availability in the second and subsequent years can be more difficult to predict. The difference method assumes that the difference in total nutrient uptake between manure-amended and nonamended plots are attributed to the addition of manure. The fertilizer equivalent approach compares crop N uptake in manure- and fertilizer-amended plots (Klausner and Guest, 1981; Harmsen and Moraghan, 1988; Motavalli et al., 1989; Muñoz et al., 2004). The fertilizer equivalent of manure is the amount of fertilizer N required to achieve the same yield and N uptake achieved with manure. Both the difference method and the fertilizer equivalent approach assume that crop N uptake in the manure-amended, fertilizer-amended, and control plots are accomplished with the same efficiencies. However, whereas approximately half (or more) of manure N is organically bound and must be mineralized by soil microbes before becoming available for crop uptake, fertilizer N is more water soluble and potentially more readily available for crop uptake.

The stable isotope ^{15}N has been used extensively to evaluate the availability of fertilizer N to crops. The use of ^{15}N to determine the availability of manure N to crops has been studied using two approaches: (i) postexcretion ^{15}N enrichment of the NH_4 pool or (2) ^{15}N enrichment of feedstuffs, which are then fed to ruminant livestock. Whereas approximately 50 to 60% of the N in slurry is in the NH_4 form (Dittert et al., 1998), semisolid dairy manure, the most important manure type on Wisconsin dairy farms (Jackson-Smith et al., 1997), typically contains much lower amounts of NH_4 and higher amounts of organically bound N. The chemical composition and mineralization of organic N in dairy manure are not well understood. More accurate estimates of manure N availability to crops are needed if we are to expect farmers to improve manure management.

Only 20 to 30% of the N (protein) fed to a dairy cow is converted into milk, with the remaining excreted about equally in urine and feces (Castillo et al., 2000; Broderick, 2003). Fecal N can be divided into two pools: (i) endogenous N consisting of microbial products and microorganisms from the rumen, the intestine, and the hind gut, and the N originating from the digestive tract itself; and (ii) undigested feed N (Mason and Frederiksen, 1979). Rumen microbial products and other endog-

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Abbreviations: NDF, neutral detergent fiber; NDIN, neutral detergent insoluble nitrogen.

enous, organic N forms in feces may make a significant contribution to crop N requirements the year following manure application. Fecal undigested feed N mineralizes slowly in soil and is therefore unavailable to plants over the short term (Sørensen et al., 1994; Powell et al., 1999). Postexcretion labeling of the NH_4 pool would not, therefore, be an appropriate technique for the study of N cycling in soils amended with semisolid manure.

The labeling of ruminant livestock manure by feeding ^{15}N forage is expensive and laborious (Sørensen et al., 1994; Powell and Wu, 1999). However, this is the only current method for labeling the urinary and fecal N components of semisolid dairy manure. However, if urine and fecal endogenous N are the only major components that contribute to crop N requirements over the short term, then an alternative method may be available to selectively label these manure N pools in a more cost-effective and less laborious manner. This paper describes two techniques that can differentially enrich in ^{15}N the urinary N, fecal endogenous N, and fecal undigested feed N excreted by dairy cows.

MATERIALS AND METHODS

Nitrogen-15 Labeling of Forage

Corn (NK N1500) and alfalfa (Cenex 'Trailblazer') plants were enriched in ^{15}N at the University of Wisconsin Hancock Research Station (44°7′ N, 89°32′ W) on a Plainfield loamy sand (sandy mixed, mesic Typic Udipsamments) during four cropping seasons (1997–2000). Initial surface (0–15 cm) soil tests were pH 6.7 (water); organic matter, 8 g kg^{-1} (loss on ignition); and Bray P_1 and K levels of 87 and 93 mg kg^{-1}, respectively. New plots within the same field were established each of the four study years.

Starter fertilizer (6, 5, and 110 kg ha^{-1} of N, P, and K, respectively) was applied annually to corn plots (approximately 32 700 plants ha^{-1}) before planting in early June. Ammonium sulfate—12.3 atom% ^{15}N in 1997 and 10.0 atom% ^{15}N in 1998, 1999, and 2000—was dissolved in water and applied using a watering can at an equivalent rate of 75 kg N ha^{-1} during the growth period to two adjoining corn rows [5 m in length (5 m^2) in 1997, 6.65 m in 1998 and 1999, and 9.11 m in 2000] in each of three applications (total application of 225 kg N ha^{-1}). We estimated that each gram of manure (urinary plus fecal) N would require approximately 2 g of forage N (an assumed forage N digestibility of 50%). Probable alfalfa and corn silage dry matter (DM) yields, their N contents, and a target diet comprised of approximately 50% of each forage type (DM basis) were used to estimate land area and fertilizer ^{15}N requirements for each forage type. Corn plants were harvested at approximately one-third milkline (60–65% moisture), chopped to 2- to 3-cm lengths, and ensiled in PVC silos.

A 20-m^2 area of a second-year alfalfa stand was used in 1997, a 28-m^2 area in 1998 and 1999, and a 40-m^2 area in 2000. Alfalfa was fertilized with 10.0 atom% ^{15}N in the same manner as corn, at an equivalent rate of 100 kg N ha^{-1} in each of two applications (total application of 200 kg N ha^{-1}). The first application was made in early to mid-June, the day after cutting alfalfa growth to an aboveground height of 2 cm. The first alfalfa ^{15}N harvest (all ^{15}N harvests involved cutting total aboveground biomass to a 2-cm residual height) occurred approximately one month thereafter. The second fertilizer application was made immediately after the first ^{15}N harvest. The second ^{15}N harvest occurred 5 to 6 wk after the first ^{15}N harvest. No further fertilizer applications were made. A third alfalfa ^{15}N harvest was taken in the fall, before first frost, in mid- to late October. All alfalfa was dried to make hay. Irrigation was applied as necessary to corn and alfalfa by central pivot.

Nitrogen-15 Labeling of Dairy Urine and Feces

Two methods were used to differentially enrich dairy urine and fecal N components in ^{15}N (Fig. 1). The *forage method* involved labeling alfalfa hay and corn silage and then feeding these forages to dry dairy cows (Powell and Wu, 1999). This technique labeled urine N, fecal endogenous N, and fecal undigested feed N. The *urea method* involved directly feeding ^{15}N-enriched urea to cows with unlabeled forage. This technique only labeled urine N and fecal endogenous N. No labeled undigested feed N in feces was expected using this technique since no ^{15}N forage was fed.

For each labeling method, two different ruminally fistulated nonlactating Holstein cows weighing 440 to 520 kg were used each year of the study. Dry cows were used to prevent loss of expensive ^{15}N in milk and in the excreta we would not be able to capture during milking. Tradeoffs in ^{15}N loss (likely 30–40% ^{15}N fed) were deemed too great compared with possible subtle differences in excreta between mature dry cows and lactating cows.

The cows were kept in adjoining stanchions and bedded with rubber mats. For both labeling methods, cows were first adapted to a diet consisting of approximately 55% alfalfa hay and 45% corn silage on a DM basis (atom% ^{15}N at natural abundance) for 7 d. On the last day of the adaptation period, indwelling catheters were inserted into the bladders for urine collection.

For the forage method, ^{15}N-enriched alfalfa hay from each harvest and corn silage (Table 1) were divided into 6 to 10 equal parts (to assure uniform ^{15}N feeding) on a weight basis. Alfalfa and corn harvested from the ^{15}N-treated plots as well from border areas (15 cm from treated alfalfa plot's edge and 30 cm from each corn row end) were fed. The hay–silage mixtures were each mixed carefully by hand. Mixtures were offered ad libitum to each of the two cows until all feed was consumed, or approximately 28 to 70 h after feeding was initiated.

For the urea method, 100 g d^{-1} of urea (^{15}N at natural abundance) was sprinkled onto the unlabeled forage fed to each cow during a 7-d adaptation period. On Day 8 in 1999, a single dose of 100 g of urea containing 5 atom% ^{15}N replaced the 100 g of urea containing ^{15}N at natural abundance. On Day 8 in 2000, single 50-g doses of 5 atom% ^{15}N urea were fed to each cow every 6 h up to 48 h (8 doses cow^{-1}). Nitrogen-15–labeled urea was dissolved in approximately 100 mL of distilled water and spread evenly over the rumen contents through the cannulus (opening to the rumen cavity). These urea feed levels were adapted from the literature review of Helmer and Bartley (1971). The 100 to 200 g fed daily were well below the 300 g found to be toxic to cattle and close to the 150-g level found to enhance the voluntary intake of oat (*Avena sativa* L.) straw by dry cows. At no time did cows show symptoms of urea toxicity (Helmer and Bartley, 1971). The urea fed provided 13 to 24% of total daily N intake (N intake levels based on assumption that cows' daily consumption of forage DM was 3.5% of body weight and that the forage contained approximately 20 g N kg^{-1}; Table 1). Early trials found that milk yields were unaffected when urea comprised 11% of total dietary N (Huber et al., 1967) and were slightly depressed when levels reached 25% (Archibald, 1943).

Total feces and urine were collected at 4-, 8-, or 12-h intervals after initial feeding of ^{15}N-enriched forage or urea up to a total of 192 h (Fig. 2–5). Feces were hand-scraped from metal catchment containers fitted into the gutters. Urine was

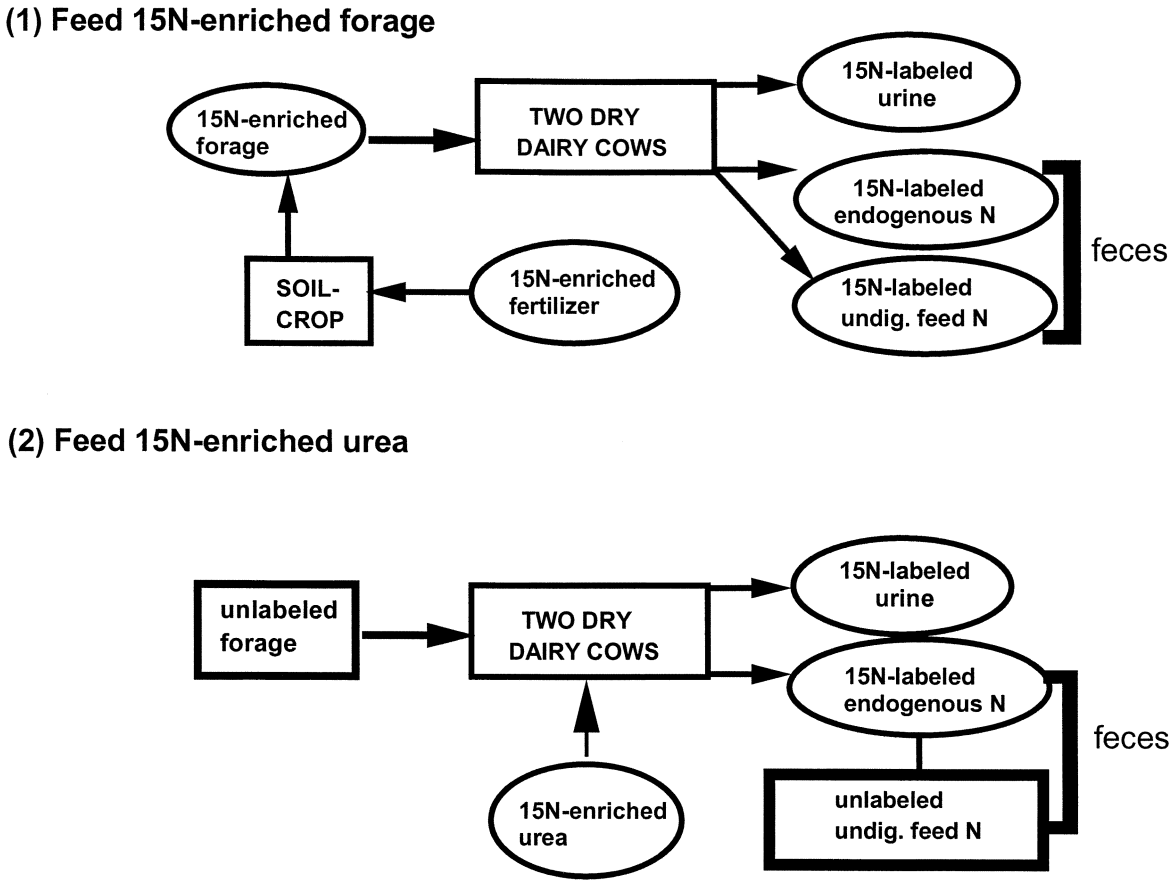

Fig. 1. Differential ^{15}N labeling of urine N and fecal N components by feeding ^{15}N-enriched forage or ^{15}N-enriched urea to dairy cows.

collected from catheter tubes draining into plastic containers embedded in ice. Feces and urine from each collection were subsampled and frozen immediately. Feces were subsampled from the metal catchment containers in 1998 and directly from the rectum at the end of each sampling period in 1999, 2000, and 2001. No apparent differences in fecal ^{15}N concentrations were noted between either technique (Fig. 3). Urine subsamples were taken from catheter tubes at the end of each sampling period.

Nitrogen Analysis

Samples of ^{15}N-enriched feeds and feces were oven-dried (60°C, 48 h) and ground to pass a 1-mm sieve for N analyses. Subsamples of ground material were dried (100°C, 24 h) for DM determination. Total N and ^{15}N concentrations in feeds, feces, and liquid urine were determined using a PDZ Europa ANCA elemental analyzer coupled with a PDZ Europa 20/20 isotope ratio mass spectrometer. Samples were flash-combusted at 1000°C and then swept through the analyzer using helium gas (Barrie et al., 1989; Barrie and Prosser, 1996).

Cell wall components of feces were determined using the detergent system (Goering and Van Soest, 1970) as neutral detergent fiber (NDF). Total N and ^{15}N contained in cell walls of feces, or the undigested feed N component in feces, were determined as neutral detergent insoluble N (NDIN). The NDF soluble N fraction in feces (endogenous N) was estimated as the difference between total N and NDIN (Mason and Frederiksen, 1979). The homogeneous ^{15}N labeling of fecal N components (Sørensen et al., 1994) was evaluated by comparing ^{15}N concentrations in total N and NDIN (Fig. 4).

The percentage recovery of ^{15}N fertilizer in alfalfa and corn and the percentage recovery of ^{15}N forage and urea in feces and urine were calculated as follows:

$$\%\ ^{15}\text{N recovered} = \frac{100P\,(c - b)}{f\,(a - b)}$$

where P is the total N amount in the forage, feces, or urine;

Table 1. Yearly ^{15}N labeling of alfalfa hay and corn silage.

Forage production year	Forage component	Component production	Total N content	Atom% ^{15}N excess†
		kg of DM‡	g kg^{-1}	
1997	Alfalfa Harvest 1	8.1	29.6	3.23
	Alfalfa Harvest 2	5.4	38.4	3.05
	Alfalfa Harvest 3	1.7	43.1	1.96
	Corn silage	12.2	8.9	6.44
	Total forage§	27.4	23.0	3.56
1998	Alfalfa Harvest 1	9.3	23.8	3.64
	Alfalfa Harvest 2	6.0	33.2	4.76
	Alfalfa Harvest 3	2.4	26.8	3.12
	Corn silage	11.0	6.5	5.23
	Total forage	28.7	19.4	4.19
1999	Alfalfa Harvest 1	9.3	25.0	3.43
	Alfalfa Harvest 2	10.8	29.8	2.92
	Alfalfa Harvest 3	5.4	33.5	3.58
	Corn silage	23.2	8.7	4.69
	Total forage	48.7	19.2	3.55
2000	Alfalfa Harvest 1	15.2	31.7	1.91
	Alfalfa Harvest 2	11.9	33.5	3.79
	Alfalfa Harvest 3	4.2	41.0	1.17
	Corn silage	28.8	9.1	4.62
	Total forage	60.1	21.9	2.92

† Atom% ^{15}N measured in fertilized forage minus atom% ^{15}N measured in unfertilized forage.
‡ DM, dry matter.
§ Dry matter, total N, and ^{15}N of forage fed to two dry dairy cows the year following forage production.

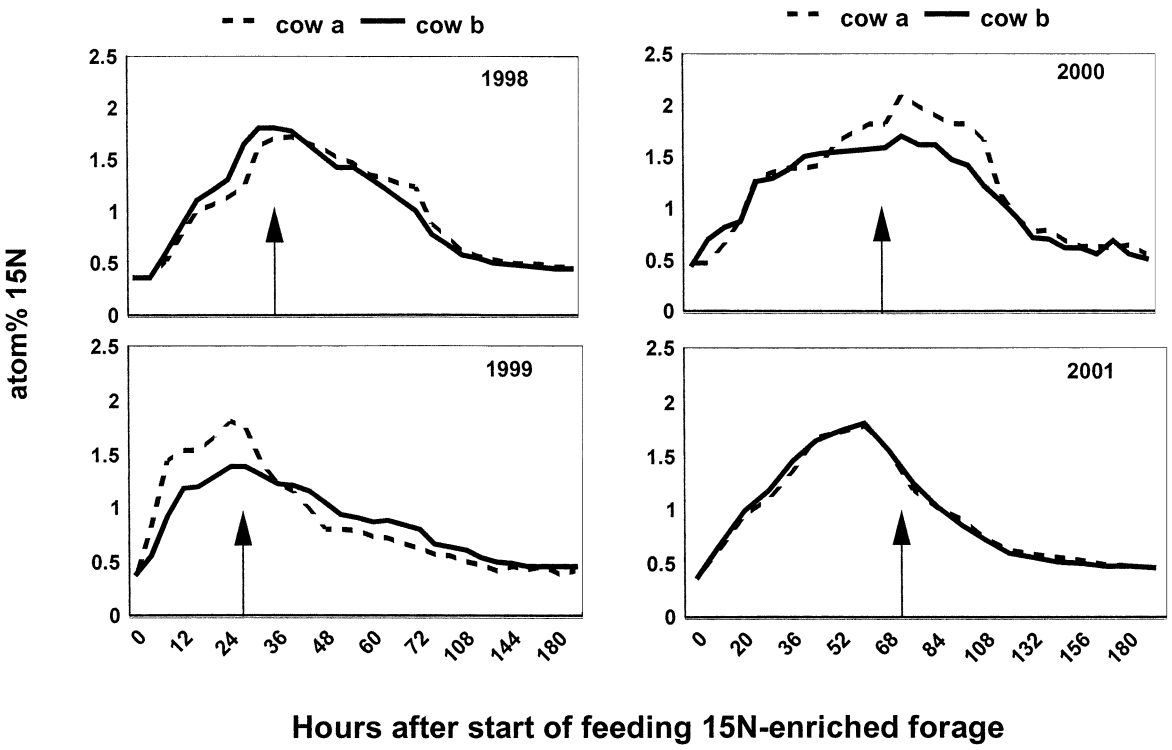

Fig. 2. Nitrogen-15 concentration in urine of two dry dairy cows after feeding ^{15}N-enriched forage. (Base of arrows marks the time when last offer of ^{15}N-enriched forage was made.)

f is the amount of ^{15}N fertilizer applied or ^{15}N fed as forage or urea; *a* is the ^{15}N in the labeled fertilizer or in the forage or urea that was fed; *b* is the natural abundance of ^{15}N in fertilizer and urea (0.366 atom%) and the ^{15}N concentration in forage recorded in unfertilized plots and in feces and urine immediately before feeding ^{15}N-enriched forage (i.e., natural abundance); and *c* = atom% ^{15}N in forage harvested from the ^{15}N-fertilized plots and in feces or urine after feeding ^{15}N-enriched forage or urea.

RESULTS AND DISCUSSION

Nitrogen-15 Labeling of Forage

Highest alfalfa yields and ^{15}N enrichments were generally attained in the first and second ^{15}N harvests (Table 1). Relatively high levels of ^{15}N enrichment were also attained in the third ^{15}N harvest even though ^{15}N-enriched fertilizer was applied only before the first and second

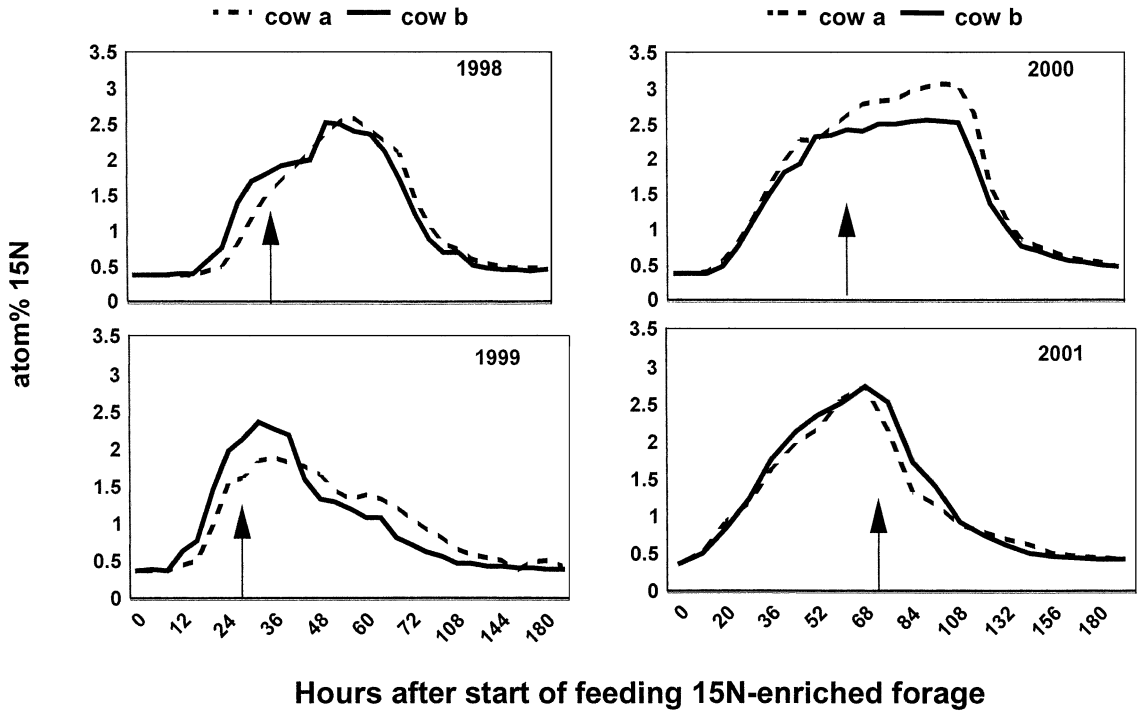

Fig. 3. Nitrogen-15 concentration in feces of two dry dairy cows after feeding ^{15}N-enriched forage. (Base of arrows marks the time when last offer of ^{15}N-enriched forage was made.)

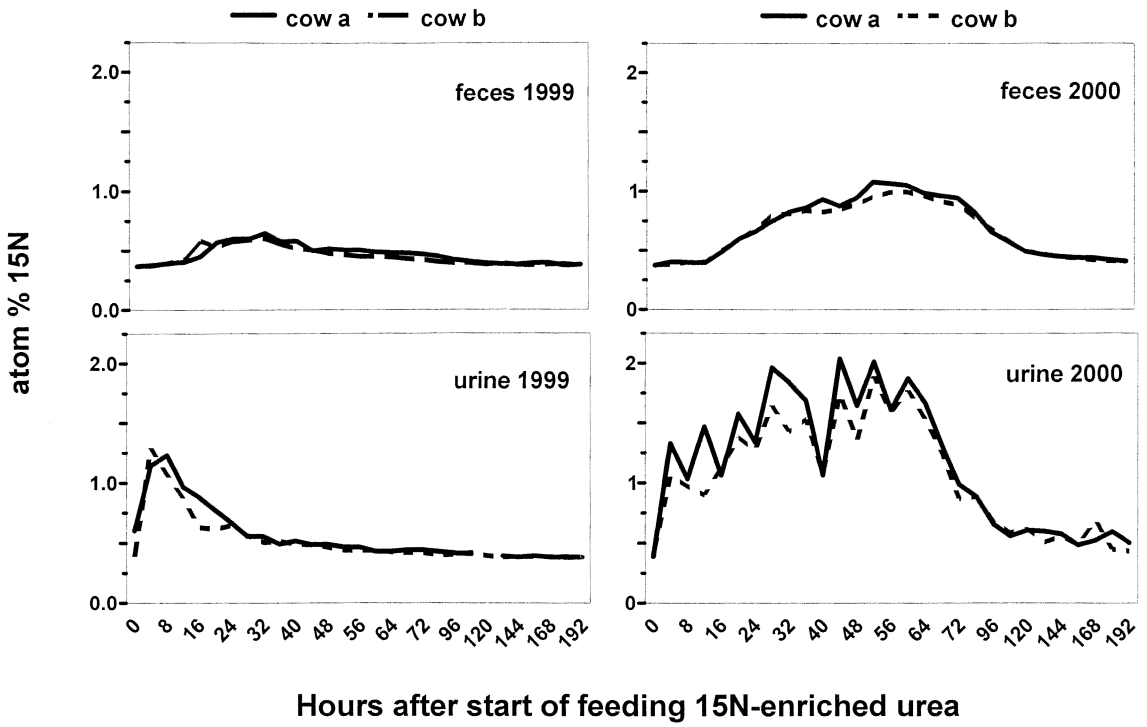

Fig. 4. Nitrogen-15 concentration in urine and feces of two dry dairy cows after feeding ^{15}N-enriched urea. (A single dose of ^{15}N-enriched urea was fed in 1999, and eight doses were fed in 2000.)

^{15}N harvests. The highest ^{15}N enrichment in corn silage was obtained in 1997, mostly due to higher ^{15}N enrichment (12.3 vs. 10.0 atom%) of the fertilizer used during that study year. The lowest ^{15}N enrichment in corn was obtained in 1998. This was likely due to abnormally high rainfall that occurred within the week after the first and second fertilizer application, which reduced yield due to leaching loss of applied N through this loamy sand soil.

For both alfalfa and corn, ^{15}N concentrations in plants harvested from the border areas of fertilized plots were four to five times greater than atom% ^{15}N in unfertilized plots (data not shown). These border row ^{15}N concentrations were approximately one-third to one-half the atom% ^{15}N measured in plants harvested from the central ^{15}N-fertilized plots. This border forage DM was, therefore, used as feed (Table 1). It provided approximately 15 to

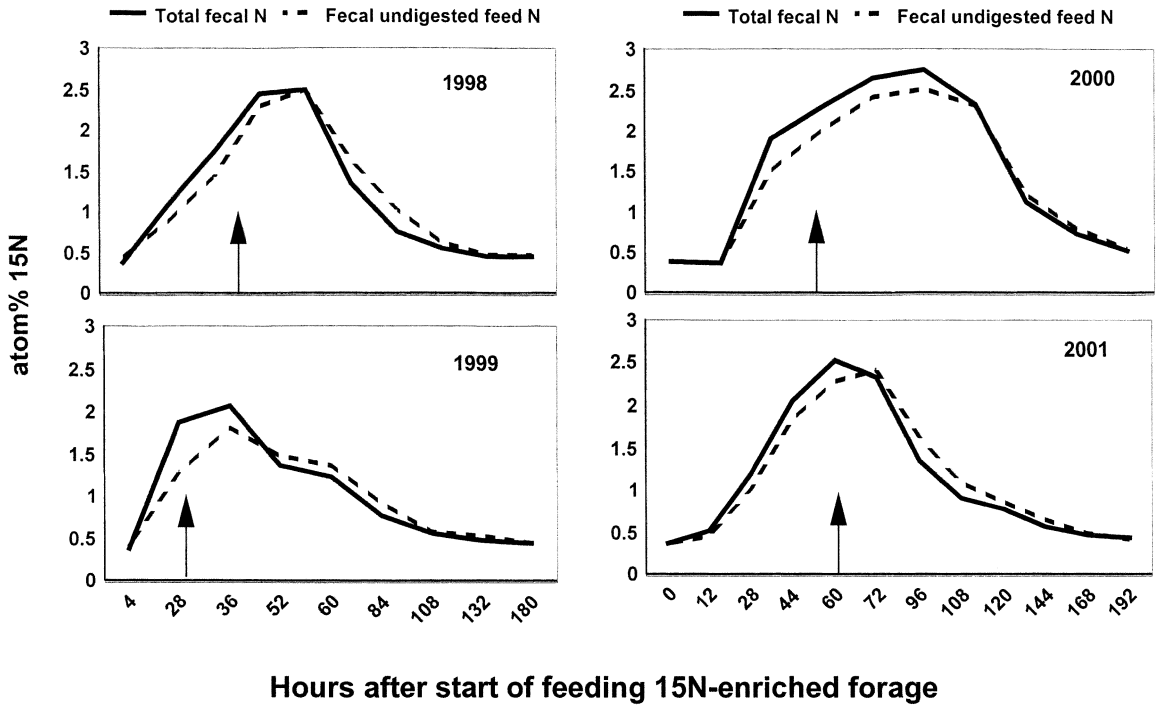

Fig. 5. Atom% ^{15}N in total fecal N and fecal undigested feed N of two dry dairy cows fed ^{15}N-enriched forage. (Base of arrows marks the time when last offer of ^{15}N-enriched forage was made.)

20% more forage than if only plot forage was harvested and fed.

Nitrogen-15 Feeding and Excretion

Both cows fed ^{15}N-enriched forage had similar patterns of ^{15}N excretion in urine and feces during all four study years (Fig. 2 and 3). Nitrogen-15 appeared in urine before feces. Except for one cow's ^{15}N excretion in feces in 2000, ^{15}N concentrations in urine and feces increased to a single maximum point and decreased thereafter. Peak ^{15}N concentrations in urine occurred between 25 and 70 h and coincided closely with the final offer of ^{15}N-enriched forage (Fig. 2). Peak ^{15}N concentrations in feces occurred between 32 and 108 h, or approximately 4 to 44 h after the final offer of ^{15}N-enriched forage (Fig. 3). Peak ^{15}N concentrations in urine and feces were attained the latest during those years (2000 and 2001) when the most ^{15}N-enriched forage was fed (Table 1). Regression analyses showed no relationship between ^{15}N concentrations in feed (Table 1) and peak ^{15}N concentrations excrete in urine (Fig. 2) or feces (Fig. 3). Sørensen et al. (1994) found that it was necessary to feed sheep ^{15}N-enriched forage for at least 7 d to achieve peak and uniform ^{15}N concentrations in feces.

The pattern of ^{15}N excretion in urine and feces after feeding ^{15}N-enriched urea (Fig. 4) was very different from the observed pattern of ^{15}N excretion after feeding ^{15}N-enriched forage. The single 100-g dose of 5 atom% ^{15}N urea fed in 1999 resulted in a single peak of ^{15}N enrichment in urine (approximately 1.25 atom% ^{15}N) 8 h after dosing and a single peak of ^{15}N enrichment in feces (approximately 0.75 atom% ^{15}N) 32 h after dosing. The eight doses of ^{15}N-enriched urea fed in 2000 resulted in eight ^{15}N peaks (from 1.25–2.15 atom% ^{15}N) in urine. Each peak was recorded within 4 h after feeding ^{15}N-enriched urea. A single ^{15}N enrichment peak in feces occurred (approximately 1.25 atom% ^{15}N) approximately 56 h after the initial offer of ^{15}N-enriched urea. Peak ^{15}N concentration in both urine and feces was attained only up to the sixth dosing. No increases in urinary or fecal ^{15}N concentrations were observed after the seventh or eighth dosing.

Nitrogen-15 Labeling of Fecal Nitrogen Components

The undigested feed N in feces (NDIN) accounted for 21% of the total fecal N, or 9% of the total N (urine plus feces) excreted by the cows in this study (Table 2).

Table 2. Concentrations of fecal undigested feed N (NDIN) in total excreted N (urine N plus fecal N) and total fecal N as influenced by ^{15}N labeling method (average of two dairy cows).

Year of feeding	Forage method		Urea method	
	Urine N plus fecal N	Fecal N	Urine N plus fecal N	Fecal N
	g NDIN kg^{-1}			
1998	95	195	NA	NA
1999	95	210	73	190
2000	100	225	103	225
2001	75	195	NA	NA
Mean	91	206	88	207
SE	5.5	5.3	12.6	14.4

The homogeneous ^{15}N labeling of fecal N components was evaluated by comparing ^{15}N concentrations in total fecal N to that in fecal NDIN. Fecal endogenous N was calculated as the difference between total fecal N and NDIN. Nitrogen-15 concentrations in NDIN were generally lower than in total fecal N during the period before maximum fecal ^{15}N concentrations were attained and higher after maximum fecal ^{15}N concentrations were attained (Fig. 5). This differential labeling of fecal N components requires that one of two strategies be used to obtain uniformly labeled fecal N components: (i) proportionately combining feces from excretion periods before and after peak fecal ^{15}N concentrations (Powell and Wu, 1999) or (ii) feeding ^{15}N-enriched forage for a longer period and using feces sampled after 15 to 20 d (Sørensen and Jensen, 1998). The latter observation was made in a trial involving feeding ^{15}N-enriched forage to sheep. Feeding ^{15}N-enriched forage to dairy cows for 2 to 4 d was very expensive (Table 3). Feeding for a longer period, such as 15 to 20 d, to obtain uniform ^{15}N distribution in feces would be cost prohibitive.

Uneven ^{15}N labeling of fecal N components may cause significant errors in estimating the rate and amount of fecal N mineralized in soil (Sørensen et al., 1994). For example, manure having a greater labeling of fecal endogenous N than undigested feed N may appear to mineralize more rapidly in soil than feces having uniform labeling. Feces having a greater labeling of undigested feed N than endogenous N may appear to mineralize slower in soil than fecal components labeled similarly (Jensen et al., 1999). However, the proportionate ^{15}N labeling of fecal N components would perhaps be more important in long- rather than short-term manure N cycling studies. The undigested feed N in feces, having already undergone degradation by ruminal microorganisms, is relatively recalcitrant in soil. After 18 mo of decomposition, 94% of labeled undigested feed N in sheep feces was recovered in the upper 10 cm of soil (Sørensen and Jensen, 1998). Various other studies have found that the NDIN in ruminant feces does not mineralize to any appreciable extent during the year following application to soils (Sørensen et al., 1994; Somda et al., 1995). Undigested feed N in feces would, however, likely play an important role in soil organic dynamics and crop N availability in fields that repeatedly receive manure.

The ^{15}N distribution in urine and feces using the urea method could have been influenced by feeding conditions, such as the amount of urea fed, protein content, and digestibility of the feed, which would influence how much urea is assimilated by microbes and how much is excreted in urine. Most studies of feeding urea to ruminants indicate that the utilization of urea N is inferior to that of conventional protein supplements. The limiting factor is rapid urea hydrolysis with much of the NH_3 absorbed from the rumen before microorganisms can incorporate it into microbial protein (Helmer and Bartley, 1971). Excess NH_3 is excreted in the urine of dairy cows (Castillo et al., 2000; Broderick, 2003). The dietary protein level of urea-fed cows was approximately 14.4 to 16.4%, of which 1.9 to 3.9% was derived

Table 3. Inputs and outputs of ^{15}N in crop and cow components using the forage and urea methods for labeling dairy manure.

		Forage method				Urea method	
		Year of forage production-feeding				Year of urea feeding	
Parameter	Component	1997–1998	1998–1999	1999–2000	2000–2001	1999	2000
		g of ^{15}N					
Crop input	Alfalfa	38.54	53.95	53.95	72.68	NA†	NA
	Corn	13.42	14.41	14.41	19.81		
Crop output	Alfalfa	15.52	19.56	23.86	26.32	NA	NA
	Corn	7.00	3.75	9.43	12.07		
Cow input	Feed	22.52	24.32	33.29	38.39	4.85	15.56
Cow output	Feces	5.64	7.05	9.10	11.02	1.00	2.73
	Urine	5.97	7.96	10.15	13.43	2.12	9.40
		N use efficiency, % recovery of ^{15}N					
	Alfalfa	40	36	44	36	NA	NA
	Corn	52	26	65	61	NA	NA
	Cow	51	62	58	64	64	78
	Overall	22	22	28	26	64	78
		$					
Costs‡		376	373	291	325	269	277

† Not applicable.
‡ Grams of labeled urine N plus fecal N (minimum of 0.4026 atom% ^{15}N) divided by cost of ^{15}N. Cost of 10 atom% ammonium sulfate was \$1.66 g^{-1}, and 5 atom% urea was \$4.20 g^{-1}.

from urea. These dietary protein levels and the amount of urea fed were not excessive (Helmer and Bartley, 1971), so a proportionate ^{15}N labeling of urine N and fecal microbial N likely occurred.

A slight ^{15}N enrichment of fecal NDIN derived from cows fed ^{15}N-enriched urea was observed both in 1999 and 2000 (data not shown). This result was unexpected and, in the context of this study, has no apparent theoretical basis for which it could be explained. No ^{15}N-enriched forage was fed, and therefore, no ^{15}N enrichment of fecal NDIN should have been detected. This ^{15}N contamination of NDIN was likely due to the inability of neutral detergent solution to remove ^{15}N-enriched bacterial nucleic acids and cell walls and glycocalyces on undigested feed fiber. Mason (1969) has shown that NDF from ovine feces retained 5 to 38% of fecal diaminopimelic acid (a bacterial cell wall component). Approximately 80% of the microbial protein is true protein, and the remaining 20% is associated with nucleic acids. Of the true protein, 80% is considered digestible (Natl. Res. Counc., 2001). Thus, 36% of the microbial protein could be excreted in feces, and this fraction would be partitioned into NDIN. However, the proportion of the microbial residual N as ^{15}N would be small as the contribution of labeled urea to microbial protein was small.

Further contamination of the NDF with glycocalyx (glycoprotein encrustations produced by bacteria to facilitate attachment of cells to fiber and other solid surfaces) is likely as these materials are often poorly soluble in detergents (Gibson et al., 1999; Landa et al., 1999; Merritt et al., 2000). We further washed urea-derived feces in acid detergent solution to remove heteropolysaccharides, the major constituents of glycocalyx structures, but even this treatment did not result in complete ^{15}N removal. This is not surprising, in view of the tenacity with which gastrointestinal bacteria are known to attach to fiber particles (Costerton et al., 1987). The continued ^{15}N enrichment of fecal acid detergent insoluble N in 2000 (data not shown) indicates that the detergent system may not be an adequate procedure for fractionating fecal N into microbial and undigested feed components.

Using Nitrogen-15 to Label Dairy Manure Components

The selection of a manure ^{15}N labeling technique depends on the intended use of the ^{15}N-labeled manure and associated costs and labor. Nitrogen cycling studies involving long-term (more than 2 yr) turnover of manure N in soils may require that urine N and both fecal endogenous and undigested feed N be labeled using the forage method. In a 3-yr field trial, recovery of applied dairy manure ^{15}N (enriched using the forage method) in harvested corn silage averaged 18%, and approximately 46% of applied manure ^{15}N remained in upper 90-cm soil profile (Muñoz et al., 2003). One of the conclusions of this study was that although costly and time-consuming to prepare, the use of ^{15}N-labeled manure using the forage method provided a much better approach to study the fate of manure N within the soil–crop system compared with unlabeled manure.

On many dairy farms, the straw of a small-grain crop is used as bedding, and this, therefore, is a manure N component. Many other low-N bedding sources (e.g., sand) are used. Nitrogen-15-labeled straw bedding has been found not to contribute significantly to crop N uptake over the short term (Jensen et al., 1999). However, this manure N component, like the undigested feed N in feces, may make a significant contribution over the long term to crop N requirements and be an important component of organic matter in soils amended often with manure.

Of the total ^{15}N-enriched ammonium sulfate used in this study, 22 to 28% was incorporated into feces and urine using the forage method. Of the total ^{15}N-enriched urea fed to dairy cows, 64 to 78% was incorporated into feces and urine (Table 3). This difference in ^{15}N use efficiency between the two ^{15}N labeling techniques was due to the loss of ^{15}N in soil when labeling forage. Of applied ^{15}N, 36 to 44% was taken up by alfalfa and 26 to 65% by corn. The lowest ^{15}N uptake by corn occurred in 1998 when high-rainfall events following the second and third fertilizer applications likely resulted in ^{15}N

leaching losses. Of the total ^{15}N fed, 51 to 64% was recovered in feces and urine from cows fed ^{15}N-enriched forage vs. 64 or 78% recovery from cows fed ^{15}N urea. Higher ^{15}N recovery using the urea method was likely due to less input to the cows and different uptakes by body tissue.

The forage method is much more laborious and costly than the urea method. The forage method requires ^{15}N application to field plots and forage harvest, handling, storage, and feeding (Fig. 1). Nitrogen-15-labeled urea can be mixed directly with forage and fed. In this study, ruminally fistulated cows were available to allow ^{15}N-enriched urea to be applied directly into the rumen via cannula. Our observations during the urea adaptation period were that urea could be just as well mixed with water and sprinkled over (unlabeled) forage and fed. However, the feeding of urea in this way may result in less recovery due to feed refusals than if urea was applied directly into the rumen.

The cost of the isotope to label urine and both fecal N components using the forage method ranged from $291 to $376 per gram of labeled manure N, and to label urine and fecal endogenous N using the urea method, the cost ranged from $269 to $277 (Table 3). These estimates were based on manure produced over the entire 8-d collection period (Fig. 2–4) using only feces and urine that contained a minimum atom% ^{15}N level of 0.403, or approximately 10% over natural abundance. Feces and urine of the highest enrichment would be needed for long-term N cycling studies, and lower enrichments could be used for shorter-term studies. Dairy manure containing at least 0.566 atom% ^{15}N would be needed to track manure N uptake by corn over a 3-yr period (Powell and Wu, 1999).

SUMMARY AND CONCLUSIONS

Manure ^{15}N labeling provides a research tool for direct measurement of N flow in various components of the feed–dairy cow–manure–soil/crop continuum. The forage method should be used to label manure for use in long-term N cycling studies as both fecal endogenous and undigested feed N components will be ^{15}N enriched. Uniform labeling of fecal N components can be achieved by the proportionate combination of feces excreted before and after peak ^{15}N excrement levels are obtained. Feeding ^{15}N-enriched forage to dairy cows over a longer period to obtain uniformly labeled fecal N components would be cost prohibitive. The urea method is less laborious and costly and may be used to label manure for short-term studies, for example, to determine crop uptake of manure N during a single cropping season.

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